Effectual comparison of quinoa and amaranth supplemented diets in controlling appetite; a biochemical study in rats

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Abstract The objective of this study was to assess the efficacy of two current cynosure protein substitutes; quinoa and amaranth in controlling short term food intake and satiety in rats. Experimental rats were allotted to three groups ($n=8$ per group) and fed with diets containing casein, quinoa and amaranth as major protein sources, with casein diet kept as control. At the end of the experiment it was observed that the rats ingesting quinoa and amaranth supplemented diets exhibited lesser food intake ($p<0.01$) and lesser body weight gain significantly in amaranth ($p<0.05$) as compared to control. They seemed to bring down plasma ghrelin levels while meliorating plasma leptin and cholecystokinin (CCK) levels postprandially ($p<0.01$). Although both quinoa diet and amaranth diet were effective in improving blood glucose response and maintaining plasma free fatty acids (FFA) and general lipid profiles subsequently after the meal, amaranth diet showed significant effects when compared to control and amaranth diets. There was 15 % improvement in blood glucose profile in the amaranth group with respect to the control at 90 min, where as there was only 3.4 % improvement in the quinoa group. These findings provide a scientific rationale to consider incorporation of these modest cereals in a diet meant to fight against growing obesity and poverty.

Keywords Quinoa · Amaranth · Food intake plasma glucose · Hormones · Rats

Introduction

The current scenario of ever-increasing obesity in the well developed countries owing to sedentary lifestyle and poverty/lack of nourishment in under developed countries present to us the irony of appetite control, which will be the savior in near future for two extreme phenomena. This challenge thrown at the scientific world is well received and currently there is burgeoning research with a ‘thirst to quench hunger’. At present there is considerable interest in the consumption of alternative crops such as buckwheat, oats, barley, spelt, rye as potential recipes for healthy food having special dietary uses (Skrabanja et al. 2001a; Di Cagno et al. 2004). The use of these pseudo cereals is of great nutritional interest because of their peculiar composition (Skrabanja et al. 2001b; Abdel-Aal and Hucl 2002; Gabrovska et al. 2002; Kim et al. 2004). Our study is one such contribution which aims at comparing two protein rich pseudocereals quinoa and amaranth, in effectively controlling short term food intake and satiety signals in rats. Quinoa is grown in a wide range of environments in the South American region (especially in and around the Andes) The distribution starts from Narino to the Salares of southern Bolivia that includes countries like Ecuador, Peru and northern Argentina (Jujuy and Salta provinces). Amaranth is the third most important staple crop for pre-Colombian people. Recently, interest in amaranth has increased because of its nutritional and functional values. Amaranth is one of the few cultivated plants from which the leaves are used as a vegetable and the grain as a cereal (Saunders and Becker 1984; Breene 1991) Generally diet rich in proteins are known to improve satiety by their thermogenic effects (Anderson and Moore 2004; Weigle et al. 2005). The amino acid composition of quinoa has been studied and quinoa proteins are particularly high in lysine, the limiting amino acid in most cereal grains Their essential amino acid balance is excellent because of a wider amino acid range than in cereals and legumes (Ruales

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and Nair 1992), with higher lysine (5.1–6.4 %) and methionine (0.4–1 %) contents (Bhargava et al. 2003; Prakash and Pal 1998). Mahoney et al. (1975) reported the protein efficiency ratio (PER) values for quinoa protein and found that the protein quality of cooked quinoa was like that of casein. Ranhotra et al. (1993) also concluded that the quality of protein in quinoa equals that of casein. Both quinoa and amaranth are consistent in their nutritive value for a fact that their protein content is very similar and comparable to the whole milk protein casein (Escudero et al. 2004; Abugoch et al. 2008; James and Lilian 2009). They also contain good amount of dietary fiber, which are known to affect satiety by their water retaining capacity. They are known to be drought or famine crops because they are cultivated with modest amount of natural resources available, but are packed with nutrition which makes them economically compatible as well (Bhargava et al. 2006).

Variability among dietary components distinctly and effectively promotes varied physiological responses. Some foods may be more effective than others in reducing appetite and prolong the intake of subsequent meal. This is mainly due to the influence of macronutrients comprising the meal. These incoming macronutrients are sensitive to the peripheral signals given out by our body, be it starvation or satiety. These signals help to orchestrate information along the gut brain axis for optimal use and storage of nutrients from the diet.

The satiety mechanism could follow different ways, including the plasmatic secretion of satietogenic proteins or peptides and transport the satiety signal from the gastrointestinal system to the brain. Two such peptide hormones having a role in the regulation of food intake are CCK and leptin, both appetite suppressants. Ghrelin, also known as orexigenic or hunger inducing hormone is the peptide hormone having a role in the short-term regulation. A growing body of data supports the hypothesis that ghrelin directly regulates glucose homeostasis and is known to cause an increase in plasma glucose in rodents (Reimer et al. 2003; Dezaki et al. 2004). Whereas leptin inhibits appetite through its action on the hypothalamus especially the arcuate nucleus (ARC). It binds to the receptors in hypothalamus to achieve the anorexic effects caused by hypothalamic neurons (Kalra et al. 1999). Circulating leptin level is directly proportional to the body fat mass and is hence regulated by fat signals like triglycerides. Leptin promotes triglyceride hydrolysis and FFA oxidation and inhibits FFA synthesis (Steinberg et al. 2002; Reidy and Weber 2002), therefore decreasing triglyceride levels. But higher than necessary levels of triglycerides may cause many health problems. This is because triglycerides bind to leptin making the leptin invisible to the brain by preventing it from crossing the blood–brain barrier. (Banks et al. 2004)

Taking into account the complex interplay of various orexigenic and satietogenic signals and subsequent unfolding of blood variables synthesised/metabolized for the regulation

of energy homeostasis, the primary working hypothesis of this study was that the satiety effect of quinoa and amaranth will be determined by the biological response patterns of key mediators of short term and long term food intake. These two protein rich cereals were compared to the effects of conventional milk protein, casein on the same set of parameters.

Materials and methods

Materials

Quinoa and amaranth seeds were purchased from a health food store and ground to fine powder prior to mixing with the diet. Mineral mixture used in the preparation of diets was procured from SRL chemicals (code: 1940128) and follows U.S.P.XIV. Vitamin mixture was prepared according to the composition specified by the National Institute of Nutrition, Hyderabad for animal diets, in their manual for laboratory techniques. Kits to analyse plasma hormones were purchased from BioVendor Laboratorni medicina a.s, Czech Republic. Kits to determine the lipid profile (triglycerides, total cholesterol, HDL, LDL) was purchased from Agappe diagnostics, India. All other chemicals used were of the analytical grade.

Preparation of diet

Diets were formulated according to the following scheme given in Table 1. The protein source for all three diets i.e. casein for control, quinoa flour for quinoa diet and amaranth flour for amaranth diet was fixed at 20 % of total dietary constituents. The protein, fat, fiber content and energy density of all three diets are presented in Table 2.

Since the present study emphasizes on the effect of macronutrient composition and its effect on appetite regulating bio molecules, slight variations in the energy density of three diets were not taken into account.

Table 1 Scheme for the preparation of animal diet

Ingredients	Quantity in %
Corn sarch	68
Casein/Quinoa/Amaranth	20
Vitamin mixture	02
Mineral mixture	04
Groundnut oil	05
Cod Liver oil	01
Dextrose	04
DL-methionine	0.3
α tocopherol	0.01

Table 2 Energy density, total amount of protein, fat and fiber in the diets

	Control	Amaranth	Quinoa
Protein(g/kg diet)	124.4	135.4	140.2
Fat (g/kg diet)	24.6	23.1	23.5
Fiber (g/kg diet)	23.4	24.4	25.0
Energy(kcal/g diet)	3.71	4.41	4.60

Animal studies

All experimental protocols were approved by the Animal Experiments Ethical Committee. Male rats of the Wistar albino strain (from in-house animal stock colony, DFRL, India) weighing between 100–120 g were maintained under hygienic conditions and kept on a standard rodent diet. A total of 24 animals were weighed and assigned to 3 groups of 8 animals each. Assignment of rats to 3 groups was made in such a way that each group had mean and total body weights similar to the other groups. Prior to the start of the experiment all the rats were housed individually in stainless steel mesh cages with individual food cups for weighed diets and they were housed in light controlled room (12 h light / dark cycles) with free access to drinking water. All the animals were maintained on a control diet for 7 days as an acclimatization period and then regrouped based on their feeding pattern. Group I was fed casein diet and kept as control, while the other two groups received quinoa and amaranth diets respectively. Food intake was recorded on a daily basis where as weight gain was calculated as an average weight gained by each rat per week.

Blood and plasma sampling

At the end of 15 days, rats were fasted overnight and on the following day, blood was collected by ocular puncture using heparinised capillary tubes. Both fasting and postprandial blood samples were obtained. Plasma was separated by centrifuging the blood samples at 2500 rpm for 15 min and stored at -80°C until further analysis.

Estimation of blood glucose

Blood sampling was done at regular intervals by snapping the tip of the tail of the rats. ARKRAY digital glucometer was used to determine both fasting and postprandial blood glucose response. The glucose concentrations were given as mg/dl of blood.

Estimation of Ghrelin, leptin and cholecystokinin in plasma

The BioVendor Rat Unacylated Ghrelin ELISA is based on a double-antibody sandwich technique. The wells of the plate supplied with the kit are coated with a monoclonal antibody specific to the C-terminal part of ghrelin. This antibody will bind to any ghrelin introduced into the wells (standard or sample). The acetylcholinesterase (AChE) - Fab' conjugate which recognizes the N-terminal part of unacylated ghrelin is also added to the wells. This allows the two antibodies to form a sandwich by binding on different parts of the rat unacylated ghrelin. The sandwich is immobilized on the plate so the excess reagents may be washed away. The concentration of the rat unacylated ghrelin is then determined by measuring the enzymatic activity of the immobilized AChE using the Ellman's Reagent. The AChE tracer acts on the Ellman's Reagent to form a yellow compound. The intensity of the color, which is determined by spectrophotometry, is proportional to the amount of the rat unacylated ghrelin present in the well during the immunological incubation.

In the BioVendor Mouse and Rat Leptin ELISA, standards and samples are incubated in micro plate wells pre-coated with anti-mouse leptin antibody. After 60 min incubation and washing, biotin-labelled polyclonal anti-mouse leptin antibody is added to the wells and incubated with immobilized antibody-leptin complex for 60 min. After another washing, streptavidin-HRP conjugate is added. After 30 min incubation and the last washing step, the remaining conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of leptin. A standard curve was constructed by plotting absorbance values against concentrations of standards, and concentrations of unknown samples were determined using this standard curve.

DRG® Cholecystokinin (CCK) (Human, Rat, Mouse) ELISA; the immunoplate in this kit is pre-coated with secondary antibody and the nonspecific binding sites are blocked. The secondary antibody can bind to the Fc fragment of the primary antibody (peptide antibody) whose Fab fragment will be competitively bound by both biotinylated peptide and peptide standard or targeted peptide in sample. The biotinylated peptide is able to interact with streptavidin-horseradish peroxidase (SA-HRP) which catalyzes the substrate solution composed of 3,3', 5,5'-tetramethylbenzidine (TMB) and hydrogen peroxidase to produce a blue colored solution. The enzyme-substrate reaction is stopped by hydrogen chloride (HCl) and the solution turns to yellow. The intensity of the yellow is directly proportional to the amount of biotinylated peptide-SA-HRP complex but inversely proportional to the amount of the peptide in standard solutions or samples. This is due to the competitive binding of the biotinylated peptide and the peptide in standard solutions or samples to the peptide

antibody (primary antibody). A standard curve of a peptide with known concentration was established accordingly. The peptides with unknown concentrations in samples were determined by extrapolation to this standard curve.

Estimation of plasma lipid profile (triglycerides, total cholesterol, HDL, LDL)

Plasma triglycerides, total cholesterol, HDL and LDL concentrations were determined according to the protocol described by commercially available kits from AGAPPE Diagnostics, Trissur, Kerala, INDIA using Automated Chemistry Analyzer system from Erba Mannheim (EM 200), Germany.

Estimation of free fatty acids (FFA) in plasma

Free fatty acids were estimated according to the method of Falholt et al. (1973) with slight modifications in sample volume and incubation time. The extraction medium used contains chloroform-heptane-methanol mixture with a phosphate buffer (pH 6.4). This extraction mixture allows sufficient extraction of FFA from serum and contamination with interfering agents is avoided. The copper soaps of FFA are determined colorimetrically with diphenylcarbazide at 550 nm.

Statistical analysis

All data are represented as mean SD ($n=8$). One way ANOVA was carried out to determine the possible differences between control group and test groups fed with different diets. Micro cal origin software was used to analyze statistical data. Values having $p<0.05$ was considered statistically significant.

Results and discussion

Food intake and body weight

The average food intake per day was measured over a period of 15 days and body weight gain was measured

Table 3 Effect of different diets on food intake in rats

	Day 1	Day8	Day 15
Control	9.6±0.11	17.5±1.11	19.5±1.16
Quinoa	9.8±1.13	16.5±0.77	15.3±1.12**
Amaranth	9.5±1.07	15.1±1.04	13.2±0.51**

All values are expressed as mean SD; ** $p<0.01$ significantly different from control on day 15

Fig. 1 Effect of different diets on average body weight gain in rats. All values are expressed as mean SD; * $p<0.05$ significantly different from control on day 15

weekly. As seen in Table. 3, though significant ($p<0.01$) decrease in food intake was observed in both quinoa and amaranth groups as compared to control, average body weight gain (Fig. 1) was significantly reduced in amaranth group ($p<0.05$) when compared to the other two groups on day 15. These results indicate that these pseudocereals have great potential to minimize short term food intake and are able to keep a check on body weight gain upon regular consumption. The plausible reasons for reduction in food intake may be understood by studying the effect of these diets on appetitive variables as explained in further results.

Plasma hormones

The better understanding of hormonal control of appetite improves understanding of the etiology of obesity or debility. The changes in the plasma concentrations of hormones reflect an activation of hunger and a blockade of satiety in order to manage food intake. Present study focused on the response of three different appetite regulating hormones namely ghrelin, leptin and CCK. These different hormone profiles influenced the qualitative aspects of feeding behavior differently during the fasting and post-feeding period. Table 4 gives us a picture of how the three different diets influenced these hormones during fasting and postprandial circumstances. Clearly, amaranth dominated the scene when it came to regulating all three hormones significantly ($p<0.01$) while quinoa was able to significantly ($p<0.01$) manage only the postprandial CCK levels, compared to control. Ghrelin is known to be a potent stimulator of hunger, but also true is the fact that ghrelin and leptin are closely and reciprocally regulated (Williams and Mobarhan 2003; Kalra et al. 2003). Ghrelin release into the blood is dependent on the nutritional state and diet composition (Moesgaard et al. 2004; Weigle et al. 2005). From the present study it is evident that amaranth, being a part of the dietary composition can ably manipulate these two interdependent hormone levels to strike a balance between satiety and hunger. On the other hand CCK is known to delay gastric

Table 4 Effect of different diets plasma hormones

Groups	Ghrelin (pg/ml)		Leptin (pg/ml)		CCK (ng/ml)	
Groups	F	PP	F	PP	F	PP
Control	86.54±4.76	51.46±2.96	40.44±1.99	60.14±3.43	5.54±0.02	8.63±0.76
Quinoa	87.21±5.48	48.67±1.74	33.72±2.67	63.32±4.32	6.01±0.03	12.56±1.03**
Amaranth	79.58±3.76	43.19±1.53**	36.32±2.91	71.19±4.07**	7.13±0.16	14.63±0.10**

Comparing fasting (F) and postprandial (PP) appetite regulating hormone profile in rats fed with different diets with that of control. All values are given as mean SD; ** $p < 0.01$ significantly different from control post meal

emptying, leading to prolonged sustenance of food in gastric cavity thereby improving satiety feeling. The overall composition of the diet has worked in favour of CCK release postprandially, since it is majorly regulated by dietary constituents especially the protein and fiber content. (Foltz et al. 2008).

Plasma glucose and lipid profile

Oomura et al. (1964) and Anand and Pillai (1967) identified neurons within areas of the lateral hypothalamus (LH) and ventromedial hypothalamus (VMH) that altered their firing rates when plasma glucose levels changed. These areas were explored first because of their proposed roles as “feeding” and “satiety” centers respectively, making it logical that they might monitor peripheral glucose levels as a means of controlling ingestion (Grill and Bjorklund 2000). Since postprandial hyperglycemia has been recognized as an important risk factor for cardiovascular diseases, even among general healthy population (Coutinho et al. 1999), it becomes necessary to manage glucose homeostasis by diet and its constituents. In accordance with this, the present study shows that amaranth diet significantly ($p < 0.01$) brought down the postprandial glucose levels (Fig. 1), thereby restricting the food and calorie consumption. Also there was 15 % improvement in the amaranth group with respect to the control at 90 min, where as there was only 3.4 % improvement in the quinoa group. The percent improvement is given by the formula (Fig. 2)

Percent improvement

$$= (\text{Control mean} - \text{test mean} \div \text{Test mean}) \times 100$$

And anything above 12 % improvement is considered clinically significant Fig. 3 shows the overall effect of three diets on plasma lipid profile (triglycerides, total cholesterol, LDL and HDL). It is seen that amaranth diet significantly ($p < 0.01$) brought down the triglyceride levels when compared to control and quinoa diet. Whereas both quinoa and amaranth diets significantly ($p < 0.01$)

reduced cholesterol levels with negligible difference between the two when compared to control. The dietary fiber from these cereals can influence absorption cholesterol level by binding to biliary acid which may increase cholesterol catabolism and the fermentation of these fibers in colon produce short chain fatty acids which contribute to inhibition of cholesterol synthesis in liver (Hara et al. 1999). There are also reports that suggest the hypocholesterolemic effect could be due to the presence of fiber, saponins, and squalene present in these seeds (Takao et al. 2005). They also seem to equally improve the LDL and HDL levels. The unsaturated fatty acids and tocopherols in these cereals may also have contributed to the reduction in LDL levels. But there are several conflicting reports on the effect of these cereals on HDL (Escudero et al. 2004; Takao et al. 2005). Our observation was in accordance to the Escudero, where the HDL levels were found to be elevated post cereal consumption. This could be due to the wide variety of phytoconstituents present in these cereals which offer a synergistic effect in exerting hypolipidemic effects (González and Rodríguez 2011). Also, excess triglycerides influence the leptin action on hypothalamus by preventing it from crossing the blood brain barrier i.e. hypertriglyceridemia causes leptin resistance (Banks et al.

Fig. 2 Mean blood glucose concentrations with respect to time in rats fed with three different diets

Fig. 3 Effect of three different diets on lipid profile (total cholesterol, triglycerides, HDL, LDL) in rats

2004). Hence the down regulation of triglycerides by amaranth diet could have significantly contributed to its effect on leptin profile and its effect of food intake thereafter. These findings suggest that, the lipid profile is reflective of body's energy needs and therefore needs to be monitored and maintained in order to achieve energy homeostasis.

Plasma free fatty acids

As seen in Fig. 4 amaranth diet stimulated a significantly ($p < 0.01$) lower free fatty acid response when compared to quinoa and control group. The concentration of plasma fatty acids may be regulated by the utilization or release of fatty acids by a number of tissues such as adipose tissue, muscle and liver. The slow release of glucose contributes to the suppression of free fatty acids in blood, thereby lowering the serum triglycerides (Liljeberg and Bjorck 2000). Since free fatty acids have been shown to impair insulin mediated

glucose disposal and enhance hepatic glucose output, prolonged free fatty acid suppression results in improved glucose and triglyceride concentrations, both of which directly influence the factors affecting food intake.

Conclusion

In general the present results based on animal studies point towards the fact that the two pseudo cereals under focus; quinoa and amaranth have very high potential to curb appetite and deserve to be a part of our daily diet. Amaranth especially managed to contribute in all the major factors responsible for regulating food intake, which was the main aim of the study. Since weight management is the primary step towards healthy lifestyle, incorporating these pseudo cereals can possibly forbid several lifestyle-related diseases like diabetes, obesity and increasing threats from such medical complications reduce short term food intake and promote healthy blood lipid levels.

Fig. 4 Mean plasma free fatty acids (FFA) concentrations with respect to time in rats fed with different diets

Conflict of interest declaration The authors declare that they have no conflict of interest including any financial, personal or other relationships with other people or organizations that could inappropriately influence, or be perceived to influence, the present work.

Submission declaration The authors vouch that the work has not been published elsewhere, either completely, in part, or in any other form and that the manuscript has not been submitted to another journal, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere without the written consent of the copyright-holder. The submitting author certifies that all coauthors have seen a draft copy of the manuscript and agree with its publication.

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